X. DETERMINATION OF THE MINIMUM DOSES OF SOME FRESH CITRUS FRUIT JUICES WHICH WILL PROTECT A GUINEA-PIG FROM SCURVY, TOGETHER WITH SOME OBSERVATIONS ON THE PRESERVATION OF SUCH JUICES.

BY ALICE JANE DAVEY.

From the Department of Experimental Pathology, Lister Institute.

(Received January 5th, 1921.)

THE following account deals with work relating to the preservation of fruit juices over long periods, in such a way as to retain the anti-scorbutic principle as little changed as possible.

The work has been carried out under the direction of Dr Harriette Chick, to whom and to Miss Hume I am indebted for much help and advice during the progress of the experiments.

Many experiments by other workers at the Lister Institute have also been included. Some of them have been published already in a summarised form [Chick, Hume and Skelton, 1918, 1, 2], for others I am indebted to my various colleagues at the Lister Institute.

INTRODUCTION.

Early in 1917, in the course of experiments concerned with the relative anti-scorbutic values of fruit and vegetables, particularly with a view to the needs of the Army, official samples of preserved lime juice, as supplied to the Army and Navy, were tested. The results showed that such preserved lime juices were practically devoid of anti-scorbutic principle, since the largest doses (10 cc. daily), that could be administered, did not indicate the slightest protection of guinea-pigs from scurvy, whereas a very much smaller dose (1.5 cc. daily) of fresh orange or lemon juice will suffice to keep a guinea-pig in good health.

It was thought that the failure of the lime juice might be due to deterioration induced by long keeping or by the method of preservation. Accordingly, tests were instituted on samples of crude juice supplied by the manufacturers and on fresh juice expressed in the laboratory from imported fruit. It was found that the fresh lime juice possessed not more than one quarter of the value of fresh lemon juice [see Chick, Hume and Skelton, 1918, 1, 2].

This result raised the question of the generally received belief in the efficacy of lime juice as a preventive of scurvy and investigations were set on foot to determine the relative anti-scorbutic values of the commoner Citrus fruit juices, and the extent to which these values were diminished by the various methods of preservation used and by long keeping at different temperatures. Attempts have been made to find a method of preserving lemon juice which shall reduce this deterioration to a minimum.

There have already been published the results of the enquiry just referred to, by Chick, Hume and Skelton [1918, 2] into the relative content of antiscorbutic principle in lemons (Citrus medica var. limonum) and limes (C. medica var. acida) where the summarised results include experiments with both guinea-pigs and monkeys. Both sets of experiments clearly demonstrate that "the value of fresh lemon juice is approximately four times that of fresh lime juice" and that preservation and long keeping of lime juice induce considerable loss of its anti-scorbutic principle.

The conflict between experimental results on the one hand and human tradition and practice on the other was resolved by the historical enquiry made by Mrs Henderson Smith [1918, 1919], regarding the use of lime and lemon juice in connection with human scurvy.

More recently, the complete failure of lime juice to afford protection to the army in Mesopotamia is a well-known fact [Willcox, 1920] and later still in Febuary and March 1919, Stevenson [1920] made the same observation of its worthlessness, among the inmates of the Russian civil prisons at Archangel.

PREVIOUS EXPERIMENTAL WORK ON CITRUS FRUIT JUICES.

Old records contain many instances of the great value of oranges and lemons for the prevention and cure of scurvy. One of the most striking is the classical human experiment carried out by Dr Lind [1757; quoted in the Medical Research Committee's Report, 1919, p. 39] by which he showed that oranges and lemons were far superior to other remedies, current in his time.

Some experiments have been carried out by Holst and Frölich [1912] on the anti-scorbutic value of lime and lemon juices, using guinea-pigs as experimental animals. These workers compared freshly squeezed raw lemon juice with commercial samples of lime juice preserved in various ways. The animals were fed on a basal diet of grain and water only; the dose of fruit juice was 5 to 10 cc. daily at most. Comparison was made with other animals receiving no anti-scorbutic. Very little protection was afforded from scurvy and the juice of fresh lemons was found little if at all superior to the preserved lime juices. The addition of the juices to the diet of grain and water prolonged life and mitigated the scurvy symptoms slightly, hence anti-scorbutic in small amount was assumed to be present in the doses given. Holst and Frölich regard this result as lending additional support to the view that guinea-pigs are more susceptible than man to scurvy, and point out that a daily dose of 5 to 10 cc. of lime juice for a guinea-pig, if related to the body weight, is a

proportionally much greater dose than that held to be sufficient to protect a man from scurvy. Since the slight protective effects of the preserved lime juice were not found to be markedly inferior to those of the fresh lemon, the authors inferred that lime juice retains most of its potency after preservation and keeping, and they assume that their experiments justify the widespread belief in the usefulness of preserved lime juice, which they never seem to have called in question.

On the basal diet of grain and water used by Holst and Frölich, with addition of anti-scorbutic in the form of lemon juice, the animals grew very little and hunger symptoms were apparent, since the animals failed to consume the grain. Some of the earliest experiments of Chick and co-workers (Chick and Hume, 1917], showed that if guinea-pigs received a basal diet of oats and bran and water with anti-scorbutic in the form of fruit juice, good health and growth could not be maintained. The animals failed to consume sufficient food and often could not be induced to take their ration of lime or lemon juice without ill effect. The diet cannot be considered equivalent to one in which a ration of fresh cabbage (containing all the accessory food factors) takes the place of the fresh fruit juices. Hence no conclusion drawn from such experiments as to the relative anti-scorbutic potency of, for instance, cabbage and lemon juice can be regarded as valid.

SCOPE OF THE PRESENT PAPER.

The present paper deals first with the determination of the minimum doses of fresh fruit juices, orange, lemon and lime, required to prevent scurvy in guinea-pigs. The results have been used as a basis for finding the loss in anti-scorbutic value which takes place when these juices are preserved or kept for long periods at different temperatures.

Besides an investigation of the keeping properties of carefully preserved laboratory material, the results are also included of tests on several commercial and official samples. Since the true history and mode of preparation of these is not always known the results lack scientific value, but are of very great importance from the practical standpoint.

Some data are also included for the value of chilled fruit. The whole investigation is still not quite complete since few of the specially prepared specimens have been kept as yet for more than two years. Such a period of preservation is however sufficient to give some good indications of the best practical methods to adopt.

TECHNIQUE.

In all particulars the method employed is that described by Chick, Hume and Skelton [1918, 2] and also by Delf and Tozer [1918]. All the experiments have been carried out on young growing guinea-pigs (about 340 g. in weight at the beginning of the experiment) fed on a basal diet of oats and bran, to which was added a ration of 60-90 cc. of cow's milk, autoclaved at 120° for an hour. The fruit juices tested were given as daily doses, administered

by pipette. All food residues were weighed or measured so that the quantities consumed daily could be recorded. The animals were weighed three times weekly. Symptoms of scurvy were noted during life and at post mortem.

The symptoms of guinea-pig scurvy are fully described by Chick, Hume and Skelton [1918, 2] and by Delf and Tozer [1918]; see also the Report of the Medical Research Committee on Accessory Food Factors [1919].

In the present paper the presence or absence of haemorrhages is taken as the decisive symptom in diagnosing scurvy or the reverse. A condition of histological bone lesion, in absence of haemorrhages, is not taken as indicative of scurvy in the light of the results obtained by Tozer [Delf and Tozer, 1918], which show that such a bone lesion frequently also occurs in animals on non-scorbutic diets and is probably due to a deficiency of vitamin A.

In order to avoid the ill effects on the animals of large doses of acid juices, all lime juices and certain of the more acid preserved lemon juices were partly, never wholly, neutralised with solid sodium carbonate. Lime juice was rendered more palatable by the addition of cane sugar (1 g. to 10 cc. of juice). Lemon or lime juice doses, whether neutralised or not, were mixed with a little autoclaved milk from the animal's ration before administration. Neutralisation was carried out as short a time as possible before administering doses to the animals, and it is assumed that no appreciable change in the anti-scorbutic value of the ration was so induced [see Harden and Zilva, 1918].

Juices used for experiment were always kept in the Lister Institute refrigerator during experiment in order to keep them as uniform as possible throughout the experiment. The temperature of the refrigerator usually ranged from -3° to 4° . On rare occasions it went down to -5° and once rose for a week to 18° .

DETAILS OF EXPERIMENTS AND RESULTS.

I. DETERMINATION OF MINIMUM DOSES OF FRESH JUICES.

A. Lemon Juice (Citrus medica var. limonum).

Sound ripe fruit was selected, any soft or blemished specimens being rejected. The large rough skinned varieties were used and efforts were made to secure uniformity as far as possible. The fruits were halved and the juice expressed by hand on a glass squeezer and strained through coarse muslin or mosquito net with firm squeezing. Juice thus prepared was kept in the refrigerator, but not frozen, during the period of testing. For the earlier experiments [Chick, Hume and Skelton, 1918, 1] supplies were made monthly, so as to be comparable with lime juice from imported fruit, which could not be obtained more frequently. In order to eliminate possible changes due to keeping even for a short time, the juice for the present experiments was prepared fresh every week.

Experiments have been carried out in order to determine within narrow limits, the minimal daily protective dose for a guinea-pig. Early unpublished

experiments, conducted in 1917, show that 10 cc. and 5 cc. daily doses afford ample protection. The paper published in 1918 recorded the effects of 2.5 cc., 1.5 cc. and 0.5 cc. daily doses and 1.5 cc. was found to be the minimal protective dose. The present experiments, using fresher juice, also test the same three doses and reach the same conclusion.

2:5 cc. daily dose. Six animals were employed. Two of these died from illnesses other than scurvy after 33 and 37 days respectively. The remaining four animals completed the experimental time and were killed in good health. They were all four in splendid condition, but two of them which had consumed a larger milk ration (85 and 95 cc. respectively, during the last 60 days of experiment), continued to increase in weight to the end of the experimental time and attained a higher maximum weight than did the others.

1.5 cc. daily dose. Six animals were used. Of these one died after 30 days of an acute intestinal affection; no scurvy was indicated. The others completed the experimental time (90 days) in good health. The post mortem indicated protection from scurvy.

0.5 cc. daily dose. Six animals were used. All developed scurvy. Two of them were killed after 45 days. Attempts were made to cure three of the others with the juice of sweet limes (see p. 90), while the remaining one was treated with canned apricot juice.

The 0.5 cc. daily dose is manifestly insufficient, but some slight protection is indicated because symptoms in life were not diagnosed earlier than the 21st to 28th day, and the length of life in cases where no cure was attempted, was not less than 40 days.

It is concluded therefore that a daily ration of 1.5 cc. fresh lemon juice (never more than one week old) is the minimum daily dose which will protect a guinea-pig from scurvy and this datum is used for the basis for comparison with the anti-scorbutic value of other fruit juices or of different preserved lemon juices. The result of this experiment is very slightly better than that detailed by Chick, Hume and Skelton [1918, 1] where the juice used was kept 0-2 months in the refrigerator, a result which suggests an appreciable though slight loss of anti-scorbutic principle when the juice is kept for such a period even at a low temperature.

B. Lemon Juice with the Rind Oil.

Experiments on preservation which are detailed later had shown that lemon and orange juice, squeezed so as to contain some of the essential oil of the rind, would keep sweet and palatable for long periods, without addition of other preservative, in the same way as lime juice, so treated, does. Lemon and orange juices squeezed without such precaution and without any other preservative, quickly become mouldy and unpalatable. Experiments to determine the minimum dose of such material when fresh are therefore included here with the determination of the minimum doses of the other fresh fruit juices.

The material was prepared in the following way. Whole lemons were sliced by hand and minced in an ordinary kitchen mincer. The juicy pulp thus obtained was squeezed by hand through coarse muslin. The resulting "juice" is a thick creamy emulsion which sets to a soft curd, from which a small amount of clear juice slowly separates. The curd is easily broken up later. This material was bottled without any sterilising precautions and seems to keep well even when the bottles are frequently opened.

As compared with fresh lemon juice expressed without the rind oil, this material is diluted somewhat with the rind constituents, *i.e.* the solid matter of the white inner rind and its watery sap.

The material was kept in the refrigerator (about 0°) during the period of the experiment, i.e. about 100 days.

For convenience in measuring small doses of the very thick material, the total quantity required each day was diluted with an equal volume of water; twice the volume of the prescribed dose was then administered to each animal.

1.5 cc. daily dose. Four animals were used; one of these showed severe scurvy after 34 days, while another completed 90 days of experiment but showed some indications of scurvy of long standing. Two others were completely protected for 90 days. 1.5 cc. of this juice was not, therefore, quite adequate.

2.5 cc. daily dose. Four animals were used of which two died early from intestinal troubles (32 and 34 days). The two remaining animals completed the experiment in good health, without signs of scurvy at post mortem.

The minimum daily dose of this lemon juice with the rind oil (0-10 days old) which will protect a guinea-pig from scurvy is therefore between 1.5 and 2.5 cc.

C. Orange Juice.

The juice was prepared exactly as is described for fresh lemon juice. Some difficulty was experienced in obtaining uniformity owing to the variation in quality of the oranges according to the season of the year. This accounts for certain discrepancies in the results. Oranges deteriorate more rapidly than lemons and late in the season they become dry. The critical experiments on which minimum dose values have been based, were carried out early in the year (Jan. to March), using as far as possible oranges of the same variety (Denia). The juice was prepared once a week, in certain experiments twice a week, and was kept in the refrigerator between successive times of preparation.

Experiments conducted in 1917, using daily rations of 10 cc., 5 cc. and 3 cc., show that these are amply sufficient to protect a guinea-pig from scurvy. Some of these experiments are mentioned in an earlier paper from the Lister Institute [Chick and Hume, 1917]. Further experiments have been conducted and are detailed below, using 1.5 cc. and 0.5 cc. daily doses.

1.5 cc. daily dose. Three series of experiments have been conducted at different seasons of the year.

- (a) Experiment extending from May to July; three animals were used; the juice was expressed weekly. Of the three animals, one died from scurvy in 50 days; another showed "doubtful scurvy" without haemorrhages after 83 days, while the third succumbed to an intestinal illness after 63 days, but was completely protected from scurvy.
- (b) Experiment extending from March to May; four animals were used; the juice was expressed weekly. Only one animal survived 90 days. It was in excellent health and was completely protected from scurvy. All the other cases were complicated by intestinal infections. No scurvy symptoms were observed during life or at the post mortem, but as the animals died or were killed after 46, 62 and 67 days, the result is not conclusive.
- (c) Experiment extending from January to March; four animals were used; the juice was expressed twice weekly. One animal died of pneumonia after 67 days but some scurvy was found at post mortem. Two others died from intestinal disorders after 74 and 88 days respectively. At the post mortem one of these showed fragility of bones but no haemorrhages, while the other was without any signs of scurvy. The remaining animal survived 90 days and at the post mortem was found to be normal.

The above results may not appear entirely conclusive; out of a total of eleven animals six were protected from scurvy and of these four survived more than 80 days of experiment. The remaining two, as well as the other partially protected animals, were all victims of infections or illnesses other than scurvy. Experiment (c), in which oranges were at their best and the juice used never more than four days old, gives a substantially better result than the others; only in one case was there any scurvy indicated at the post mortem, and that by fragility of the bones only, haemorrhages being absent. In fact three out of the four animals were protected.

0.5 cc. daily dose. An experiment with one animal was begun in June 1918. Oranges were then dry and their anti-scorbutic potency lowered as shown by the experiments described above with larger doses. The animal died on the 58th day of experiment with moderately severe scurvy complicated by other illness.

A further experiment was begun in March 1919, the juice being prepared twice weekly. Five animals were employed, all of which showed signs of scurvy round about the 34th day of experiment. One of them showed scurvy with severe haemorrhages and fragile bones after 57 days; another died from disease other than scurvy after 43 days; scurvy haemorrhages were not found but the bones were slightly fragile and the rib junctions nodular. The remaining animals were suffering from severe scurvy from the 57th to the 67th days, as indicated by sore swollen joints, lameness etc. At this time their doses were changed to a daily ration of sweet lime juice which effected a partial cure.

From these experiments it is assumed that 1.5 cc. daily may be considered a minimal protective dose for a guinea-pig, when the freshly expressed juice of oranges at their best, i.e. in the early part of the season, is used.

D. Orange Juice with the Rind Oil.

Experiments with lemon juice with the rind oil were so promising that material was prepared in exactly the same way from oranges, in order to establish the minimum dose and test the keeping capacity of this material also.

The fruit was obtained early in March 1919; it was treated exactly as is described for lemons. The emulsion was very thick and set to a substantial curd, a small proportion of juice squeezed without the rind oil was therefore added.

The material was kept in the refrigerator at a temperature about 0° for the time of the experiment, *i.e.* 0-108 days.

1.5 cc. daily dose. Four animals were used, of which two were in good health at the end of the experiment. Two died from other causes (63 and 72 days) with very doubtful scurvy, slight bone brittleness but no haemorrhages.

3 cc. daily dose. Two animals were used and both were normal at the end of the experiment. The minimum dose of orange juice with the rind oil kept in the refrigerator for 0-108 days was therefore taken to be 1.5 cc. daily.

E. Sweet Lime (Citrus medica var. limetta).

A small sample of sweet limes was received from Basra in February 1919, sent by Lt.-Col. Ledingham. The sample arrived in poor condition owing to the long time taken in transit. Many of the fruits were useless and the remainder though placed in the refrigerator, deteriorated rapidly and became mouldy and soft. Juice was expressed every two or three days.

It was tested as a cure for animals sick with scurvy, induced by insufficient doses of fresh lemon and orange juices.

Of the six animals used, three had been on a daily ration of 0.5 cc. fresh lemon juice for 49, 52 and 48 days respectively, while three had been on a similar ration of fresh orange juice for 57, 65 and 67 days respectively. All were suffering from severe scurvy as evinced by swollen, painful joints, scurvy position, lameness and falling weight. A daily ration of 2.5 cc. of the juice of sweet limes was substituted for the orange and lemon doses. A very gradual improvement in the condition of the animals became apparent with steadying of the previously falling weight curves; in some cases slight gain in weight took place. Although the cure was very far from complete, the life of the animals was very considerably prolonged. The average life of the six animals in question was 78 days and two of them survived 89 and 91 days respectively; whereas the average length of life of five animals which continued on the small doses of orange and lemon was 49 days only. The post mortem

examination revealed some repair of bone lesions and absence of recent haemorrhages, although signs of severe old haemorrhages were apparent.

Although the sweet limes were old and in very poor condition their juice exercised considerable curative power when administered in 2.5 cc. daily doses to guinea-pigs suffering from severe scurvy. No definite conclusions can be drawn as to the value of sweet limes in good condition, but the result obtained suggests that they should be useful as an anti-scorbutic and superior in this respect to sour limes.

F. Sour or West Indian Lime Juice (Citrus medica var. acida).

The experiments with fresh ripe lime juice were conducted during the winter of 1917-18 and have been summarised by Chick, Hume and Skelton [1918, 1, 2]. The results are included again here, with some further notes for comparison with the newer results from preserved lime juice and juice from green limes.

The fruit used was obtained through the kindness of Messrs L. Rose & Co. who furnished monthly supplies of specially imported West Indian limes. Owing to war conditions these limes were from one to two months in transit. They do not seem to bear keeping as well as lemons, possibly because of their thinner rind, from which the volatile essential oil is more easily lost.

The juice was prepared exactly as described for lemon and orange juices without the rind oil, only sound firm fruit being employed in its preparation.

The experiment included tests with green unripe limes as well as with ripe limes, as it was thought that the anti-scorbutic value of the green fruit might be greater than that of the ripe fruit. We can draw no very definite line as to what constitutes the limit of ripe and unripe fruit, but ripe fruits were definitely yellow.

Experiments were conducted with 2.5 cc., 5 cc. and 10 cc. daily doses of fresh lime juice. The doses were half neutralised with sodium carbonate, and cane sugar (1 g. per 10 cc. juice) was added to render the juice palatable.

The age of the juice after squeezing varied from 0 to 30 or 40 days old, it being preserved for that time in the refrigerator between squeezings.

2.5 cc. daily dose. (a) Ripe lime. This amount of ripe lime juice afforded no protection from scurvy. Of seven animals, five died from severe scurvy in 23 to 59 days. The remaining two cases were complicated by other illness and scurvy was less severe after 29 to 67 days, than in the preceding cases.

(b) Green lime. When the juice from green limes was employed the results were appreciably better. Scurvy symptoms were milder and the duration of life was longer. One of the four animals used lived for 116 days suffering from slight chronic scurvy, with some improvement towards the end of the experiment, probably correlated with the use of a fresh sample of juice. In another animal which died from infection after 38 days, the post mortem showed no sign of scurvy. The two remaining animals showed scurvy of moderate severity after 56 and 85 days respectively.

5 cc. daily dose. (a) Ripe lime. Ten animals were used of which three showed definite scurvy and three were normal; two showed doubtful scurvy and two died from other causes (26 and 35 days) without haemorrhages.

(b) Green lime. Seven animals were used, of which three died within 34 days from other illness than scurvy and are therefore discounted. As with the ripe lime, practically complete protection was obtained in three of the four cases remaining; the animals survived for over 90 days and no haemorrhages were found at post mortem. The fourth animal showed only doubtful traces of scurvy after 56 days.

10 cc. daily dose. Ripe lime only. Doubling of the 5 cc. dose produced no appreciably better result and it failed to eliminate the slight fragility of the bones and enlargement of the rib junctions, with the corresponding histological symptoms, which so frequently occurred but which there is little doubt were due to an insufficiency of vitamin A.

Of six animals one was normal in all respects after 88 days of experiment. Another died with severe visceral haemorrhage after 85 days, showing fragility of bones at post mortem. Scurvy was doubtful or absent (no haemorrhages) in two other cases after 52 and 62 days respectively and two died without haemorrhages at 25 and 34 days from other causes.

It must be noted that animals receiving lime juice never grew as vigorously as the animals receiving orange juice or the smaller doses of lemon juice. Healthy animals usually attain a maximum weight of from 550 to 600 g. within the 90 days of experiment, their weight curve approximating nearly to that of animals fed on normal diet. With one exception the highest maximum weight reached by any animal on a 5 cc. or 10 cc. fresh lime juice ration was 470 g. The exception was the single completely protected animal on the 5 cc. daily ration of fresh ripe lime juice, whose weight reached 537 g. on the 78th day of experiment.

The experiments with fresh lime juice indicate 5 cc. daily as the minimal protective dose for a guinea-pig. Taking into consideration the poor growth and consequent low body weight of even those animals which were completely protected from scurvy, it is possible that the 5 cc. daily dose might not ensure protection to better grown animals, whose weight curve more nearly approximated to the normal.

On the other hand, conditions necessitated the use of lime juice prepared monthly from fruits which had suffered from delay in transit. If tests be made from lime juice prepared at more frequent intervals from fresh fruit, the results may indicate a slightly lower value for the minimal dose than has so far been obtained. Pending such further experiments, the minimal protective dose of fresh lime juice required by a guinea-pig cannot be fixed at less than 5 cc. daily, which amount is perhaps best regarded as marginal.

II. PRESERVATION OF FRUIT JUICES.

A. Official and Commercial Samples.

The work carried out with official or commercial samples chiefly concerns the juices of lemon and lime, on account of their use in the rationing of armies, arctic expeditions etc. and the consequent urgent need during the late war for exact information as to their anti-scorbutic value.

Tests have been carried out with a number of official samples as supplied to the Army and Navy, obtained through the authorities. Various commercial samples preserved by different methods have also been tested. Details with regard to a number of such samples have already been published [Chick, Hume and Skelton, 1918, 1]; a few more tests of special samples are added here and the tests of a certain number of similar samples of lemon juice.

Acknowledgments are here made to Messrs L. Rose & Co. and to Messrs Evans Sons, Lescher & Webb for their kindness in supplying samples and furnishing data as to age and method of preparation.

In view of the proved inferiority of lime juice to lemon juice in the fresh state and the uselessness of the preserved lime juices, and having regard also to the promising results obtained with preserved lemon juice, it was recommended to the authorities that steps might be taken to substitute lemon juice for lime juice in supplies to the Army and Navy, thus reverting to the usage of the early part of last century. In response to this suggestion, samples of lemon juices submitted to the authorities have been tested on their behalf.

Since there is some evidence that greater care in the method of preservation will allow retention of more of the anti-scorbutic potency, careful comparison has also been made of the above-mentioned lemon juice with lime juice specially prepared by Messrs Rose, on exactly the same lines as were recommended and adopted in preparing the samples of lemon juice.

A 1. Official and Commercial Samples of Lime Juice.

Reference to the table given by Chick, Hume and Skelton [1918, 1, Table I] shows that of six samples of crude lime juice examined, none showed any protection in 5 cc. daily doses, save two samples (Nos. 3 and 4 in table) which did show some small degree of protection. In the case of two of the samples however, even a 10 cc. daily dose conferred no protection.

Two further samples, Nos. 7 and 8, were examined in the course of the present work and yielded a similar result. These were both crude juice supplied by Messrs Rose; the one (No. 7) was prepared from ripe limes in the usual way save that none of the essential oil of the rind was removed before crushing and squeezing; the whole fruit was squeezed right out and the juice, containing more than the usual amount of essential oil, was run straight into the casks for export; two animals on 5 cc. and two on 10 cc. of this material all showed scurvy.

The second sample (No. 8) was ordinary crude lime juice containing no

more than the normal amount of essential oil but prepared from green limes instead of ripe ones. Four animals received 5 cc. of it daily and all showed scurvy. In neither of these cases was the juice more than 2-3 months old when the experiment started.

A third sample (No. 9) was prepared with especial care by Messrs Rose so as to be comparable with the special sample of lemon juice submitted on behalf of the War Office. The material appeared to be crude juice containing the rind oil and 0.07 % SO₂, added as preservative. This sample of lime juice gave the best result ever obtained with lime juice. The lowest value hitherto obtained for the minimum daily dose of lime juice needed to protect a guinea-pig from scurvy is 5 cc., even with juice expressed from imported, though not very fresh limes. With this dose not all guinea-pigs were protected. The special sample, at present under consideration, gave a value rather less than 5 cc., all animals on 5 cc. being perfectly protected; animals on 2.5 cc. all showed scurvy. The inferiority of lime juice is thus still upheld, though in this special case it is considerably less marked than in any other sample ever tested.

A 2. Official and Commercial Samples of Lemon Juice.

Four different samples were examined and of these three gave good results, showing a far higher degree of protection on a 5 cc. dose than did the commercial samples of lime juice.

No. 10 was a sample of lemon juice prepared by Messrs Rose for the War Office, squeezed with the rind oil and with $SO_2 = 0.1 \%$ as preservative. This sample corresponds with the lime juice sample No. 9. Protection was obtained in two cases, and a doubtful result in the third case with a 1.5 cc. daily dose of this material, a result practically equal to that obtained with the freshly expressed juice.

No. 11. Lemon juice, expressed without the rind oil, preserved in Messina with 0.25 gal. sulphurous acid to 100 gals. of juice. This juice had been kept for at least six months at room temperature. Only a 5 cc. daily dose was administered and this gave undoubted protection in three cases out of four, the fourth being uncertain. Considerable loss may therefore have taken place but it cannot have been by any means complete.

No. 12. A lemon juice sample furnished by Messrs Evans Sons, Lescher & Webb. It was received in October from lemons squeezed in the preceding spring in Sicily. No preservative was added and apparently none of the rind oil. Only 5 cc. and 10 cc. daily doses were administered and in each case no scurvy developed. Loss, if it had taken place, was therefore not great.

No. 13. A sample of "Kia-Ora" lemon squash, bought in a shop. The history was unknown. Sugar had been added. Doses of 5 and 10 cc. showed no protection from scurvy. This sample therefore ranks with the commercial samples of lime juice, all the other lemon juices being very much superior.

It is quite clear from the results so far given that lemon juice is much

more potent anti-scorbutically than lime juice. It is not however possible to make an accurate quantitative comparison of the keeping capacities of the two, for it is easy to give to guinea pigs a 5 cc. dose of lemon juice, which gives the information whether lemon juice has lost two-thirds of its anti-scorbutic value or not, but it is impracticable to give 15 cc. of lime juice, which is the daily dose necessary in order to obtain the same information about lime juice.

B. Preservation of Special Samples.

The foregoing experiments show clearly (a) that orange and lemon juice are more potent as anti-scorbutics than lime juice, and (b) that they are on this account more suitable material for use in keeping experiments where gradual loss of anti-scorbutic potency is to be anticipated.

Samples of lemon juice prepared from fresh fruit were stored in the Lister Institute refrigerator, without addition of any preservative. They were tested after an interval of seven months, and it was found that a daily dose of 5 cc. conferred almost complete protection on a guinea-pig. Such juice retained its value even after fermentation of the sugar had taken place, a 5 cc. daily dose again conferring considerable protection. This result stimulated further the enquiry into the methods of preserving and keeping lemon juice, and experiments have been carried out with juice prepared in the laboratory, while others are still in progress. As it was believed that the temperature at which the juice was stored would make considerable difference to the antiscorbutic, as well as to the gross keeping capacity of the juice, samples were stored at different temperatures. Experiments with samples of lemon juice preserved with varying percentages of sulphite and with the rind oil and of orange juice with the rind oil are also given.

B 1. Preservation of Lemon Juice by means of "Sulphite" (potassium metabisulphite).

The juice was expressed from carefully selected fruit, exactly in the manner described for fresh fruit juices. Quantities of from 20 to 30 litres were prepared at one time and the process occupied one or two days. The sulphite was added in 10 % aqueous solution, the juice being stirred vigorously to ensure complete mixing. The juice was bottled immediately, stoppers or corks being sealed with wax so as to be airtight. Two strengths of preservative were tried giving final concentrations of 0.06 % and 0.09 % sulphite in the juice. In the case of a sample of juice to be kept at a higher temperature 0.1 % sulphite was used. It has been found that juice preserved with sulphite does not keep well after bottles have once been opened, hence it is best to store it in bottles containing quantities suitable for a single experiment. Sterilising of bottles previous to filling made no appreciable difference and was accordingly dispensed with.

Samples of this material have been stored for periods of many months, under the following conditions:

- (a) At ordinary room temperature (12° to 25°), in a dark cupboard in a corridor, not subjected to sunlight.
 - (b) In a hot room at 37°.
- (c) In the Lister Institute refrigerator, where the temperature was usually slightly above 0° but ranged from -3° to 4° , once falling as low as -5° and once (owing to a breakdown) for a week rising to 18° . In winter the juice was occasionally frozen.

The preserved juice is a clear pale yellow liquid containing a small amount of curdy sediment. When efficiently sealed the material does not darken perceptibly after 12 months' keeping in the cold room or at room temperature, but in the hot room considerable darkening takes place in the course of a few weeks although the juice continues for some time to remain good as far as taste and smell are concerned; ultimately however it becomes bad and quite unpalatable. The samples to be tested were removed from the place of storage at the beginning of the experiment and kept in the refrigerator while the experiment progressed.

Tests have been carried out as shown below.

- (a) Lemon juice preserved with sulphite, kept at room temperature.
- 1. Material preserved with 0.06 % sulphite.

Two batches of this material were made. The first batch (No. 14) had been entirely used up after eight months; a second batch (No. 15) was therefore made for further keeping.

Age $3\frac{1}{2}$ to 5 months (No. 14). After $3\frac{1}{2}$ to 4 months, a 2.5 cc. daily dose failed to protect. After 4 to 5 months a 5 cc. daily dose failed to protect, but the juice appeared to have gone bad, probably due to faulty sealing and all the four animals used developed scurvy as though no anti-scorbutic had been given. On the 19th and 25th days respectively, two of them had their doses replaced by 5 cc. of the juice left over from the last experiment. This seemed to check falling weight in one case but after 14 days both the animals showed advanced scurvy, being thin and miserable. Their dose was then changed to 5 cc. of juice from an unopened bottle of the same material. After 14 days the condition of both animals improved remarkably; there was rise of weight and the use of the hind limbs was regained; they were then killed and showed good condition and repair of bone lesions.

Thus a 5 cc. daily dose, after four months' keeping at room temperature, is in some cases sufficient to effect a cure in cases of advanced scurvy. This indicates a deterioration to about one-third of the original value.

Age 6 to 10 months (No. 14). A 5 cc. daily dose gave almost complete protection. Of the four animals used, one died from disease other than scurvy in 30 days. Three animals completed the experiment (88–90 days). There

was evidence of slight scurvy, some fragility of bones and slight haemorrhage being found at post mortem.

A further test was carried out using 10 cc. daily. Only one animal was employed. It never made very good growth, remained thin and in poor condition and died after 61 days. Slight fragility of bones was found, but the animal was protected from scurvy.

None of the above-mentioned animals received more than 60 cc. of autoclaved milk daily. Animals receiving large daily rations of the juice preserved with sulphite never made good growth and failed to reach the weights attained by normal animals or by those on small rations of fresh juice. In subsequent experiments the animals received a larger milk ration and the doses of lemon juice were half neutralised with sodium carbonate.

Age 18 to 22 months (No. 15). Animals receiving a 5 cc. and a 2.5 cc. daily dose showed good health after 60 days; the experiment was unfinished at the time of going to press but it seemed likely that the 2.5 cc. daily dose would afford protection, showing that in this case after nearly two years' keeping at room temperature, lemon juice preserved with 0.06 % sulphite, in some cases at any rate, retains at least three-fifths of its anti-scorbutic potency (the average minimum protective dose of fresh lemon being 1.5 cc.).

2. Material preserved with 0.09 % sulphite.

Age 7 to 12 months (No. 16). At seven months a 5 cc. dose effected protection. At 12 months a 2.5 cc. dose failed to do so.

Age 19 to 21 months (No. 16). At 19 months a 5 cc. dose failed to protect. At 21 months two animals on a 10 cc. dose were in good health after 50 days of experiment; the experiment was still unfinished at the time of going to press.

Material preserved nearly two years with 0.09 % sulphite at room temperature therefore, in this particular set of experiments, showed signs of slow, steady deterioration down to one-fifth or one-sixth of its original value (taking the minimal dose of fresh lemon juice as 1.5 cc.). The various results with lemon juice preserved with sulphite are however rather inconsistent and the different points are more fully discussed later.

(b) Lemon juice preserved with sulphite, kept at 37°.

This material was prepared in April 1918 and contained 0·1 % of sulphite. Tests were carried out after three months' keeping, when the juice was already very dark in colour and after 28 months.

Age 3 months (No. 17). Four animals received 5 cc. daily. One of these died from infection after 30 days but it is doubtful whether scurvy was beginning. Another animal showed complete protection from scurvy after 67 days. The remaining two survived for 89 days, one with slight scurvy and the other with severe scurvy, the onset of which dated from about the 67th day.

Bioch. xv

Although insufficient for complete protection the 5 cc. dose was of considerable value.

Age 28 months (No. 17). There was no evidence of any protection when a daily dose of 10 cc. was given, the two animals used dying of scurvy in 23 to 30 days. Thus the material when kept at a temperature of 37° showed no anti-scorbutic value after two years; it was also bad in the gross sense.

(c) Lemon juice preserved with sulphite, kept in the refrigerator (about 0°).

This material contained 0.06 % of sulphite and was the same as that used as No. 14. Tests were carried out on it after 5 and 27 months' keeping.

Age 5 months. A 5 cc. daily dose afforded complete protection to four animals.

Age 27 months. A 2.5 cc. daily dose afforded complete protection to two animals. Of two animals receiving 5 cc. one was completely protected and one which became ill from other causes showed slight symptoms of scurvy; doubtless this anomaly, in which the 2.5 cc. dose appears more effective than the 5 cc. was due to the interference of intercurrent disease. In the case of this material stored for more than two years at about 0°, the anti-scorbutic loss seems very slight.

Summary of results, obtained with lemon juice preserved with sulphite.

On reference to the details (Nos. 14, 15, 16) of material stored at room temperature, it is seen that no daily ration of less than 5 cc. was capable of giving adequate protection to a guinea-pig from scurvy, except possibly in the case of the unfinished experiment with 2.5 cc., 22 months old (No. 15). After the juice had been kept only four months, the 5 cc. dose was barely adequate while half this amount was insufficient. Nevertheless, after 18 months' keeping, a 5 cc. daily dose of lemon juice containing 0.06 % of sulphite afforded complete protection.

The effect of the time factor during periods of less than three months has not been investigated. There is considerable loss of anti-scorbutic within this period, since the minimum daily dose of fresh lemon juice is 1.5 cc. daily.

There is no striking difference corresponding with the different percentages of sulphite employed, but the larger amount is probably to be recommended.

The results contain one striking discrepancy, namely that while 5 cc. of lemon juice, containing 0.06 % sulphite, constituted a protective dose after 18 months' keeping, the same dose of similar material, containing 0.09 % sulphite, was powerless to prevent scurvy. Such inconsistencies as this, considered together with the ready tendency of sulphite material to deteriorate after exposure to air, suggest that the sulphite method of preservation is unreliable, in some cases giving good results but in others failing completely.

The keeping experiments at higher temperatures were carried out in order to test the possibility of preserving lemon juice for use in hot climates. As

might be expected, the destruction of anti-scorbutic goes on more rapidly than at lower temperatures. Nevertheless the material was not entirely useless after three months' storage, when a daily dose of 5 cc. afforded considerable protection from scurvy. After 27 months' storage however, no value at all could be detected.

Preservation at lower temperatures increases the time for which the juice-will remain efficacious. Extreme limits have not yet been determined. After 27 months' keeping, a 2.5 cc. daily dose of material stored in the cold room protected a guinea-pig from scurvy.

B 2. Preservation by means of the Rind Oil.

Experience with lime juice showed that when the juice is expressed from the whole fruit so as to contain the essential oil of the rind it keeps well in the gross sense, without the addition of foreign preservatives. It seemed probable that this method might also serve for other Citrus fruit juices. Small samples of such juice expressed from minced lemons were found to keep well in the laboratory so far as taste and smell are concerned. It remained to test how far the anti-scorbutic value was at the same time preserved.

Accordingly large quantities of material were prepared for testing in the way already described under the determination of the minimum dose for orange and lemon juice with the rind oil. Arrangements were made to carry out tests at considerable periods on material stored at room temperature, at 37° and at a temperature round about 0° (in the Lister Institute refrigerator) in the case of lemon juice, and at 37° and room temperature for orange juice. Material from whatever source was, as usual, stored in the refrigerator during the actual course of each individual test.

(a) Lemon juice with the rind oil.

1. Kept at room temperature. No. 19.

Material was tested at $4\frac{1}{2}$ months, at 12 to 13 months, and 24 to 27 months. Age $4\frac{1}{2}$ months. A 2.5 cc. daily dose gave full protection showing little or no deterioration to have taken place.

Age 12 to 13 months. Both 2.5 and 5 cc. daily doses gave protection, showing that after a year at room temperature, little deterioration had still taken place.

Age 24 to 27 months. The material used in this test had not been kept for the whole time at room temperature, for the first 10 months it was kept at about 0°, later being kept at room temperature. 2.5 cc. failed to give protection. 5 cc. dose gave good health up to 60 days and promised to give full protection though the experiment was incomplete at time of publication.

Even after 10 months at about 0° and 14 to 17 months at room temperature, therefore, the loss in anti-scorbutic value of lemon juice preserved with the

rind oil though perceptible was not very great, and the result compares exceedingly favourably with the lemon juices preserved with sulphite, where only one specimen gave a similar result while other specimens kept for shorter periods failed to protect in larger doses.

2. Material stored in hot room, 37°. No. 20.

Under these conditions the juice darkened after a few weeks, but smelt and tasted good after many months. The first samples were not tested till after 24 months' keeping. Two animals received a 2.5 cc. dose, two a 5 cc. dose and one a 10 cc. dose. All showed severe scurvy and there was little if any sign of protection even in the case of the 10 cc. dose. Further experiments with this material were therefore abandoned as it was evident that the method of preservation was useless at such a temperature as 37°.

3. Material stored in cold room ("chilled"; about 0°). No. 21.

The material was tested for the first time after 26 months' keeping. Two animals receiving 2.5 cc. daily remained in good health for the full period of experiment. Lemon juice with rind oil preserved at about 0° does not therefore lose a perceptible amount of its anti-scorbutic potency even when so stored for more than two years, a property which it shares with the lemon juice preserved with sulphite when that is stored at the same temperature.

Summary of results with lemon juice preserved with the rind oil.

The juice preserved in this way at 0° and 37° gives much the same result as juice preserved with sulphite. Both show little or no loss of anti-scorbutic potency even after two years at 0° and both scarcely appear to retain any potency after preservation for two years at 37°.

It is in the results at room temperature that the material preserved with rind oil shows its superiority over that preserved with sulphite. In one case, No. 15, the sulphite material appears to be as good, but it evidently cannot be relied upon, particularly after the bottle is opened. There is no evidence that opening the bottle has any destructive effect upon the rind oil material.

(b) Orange juice with the rind oil.

The preparation of the material has already been described under the determination of the minimum dose. A portion of the material then tested was set aside for keeping and was preserved at room temperature and at 37°.

Whenever a test was started the portion of material required was kept at about 0° until after the test was finished.

1. Preserved at room temperature. No. 22.

Aged 16 to 19 months. This material was not tested till it was 16 to 19 months old.

Of two animals on a 3 cc. dose one developed scurvy towards the end of the 90 days experiment, the other died from other causes at 63 days without signs of scurvy.

On a 5 cc. dose two animals were in good health after 50 days and one after 22 days, the experiment was unfinished at time of publication but promised well and seemed to suggest that orange juice with the rind oil behaves much in the same way as does lemon juice.

2. Preserved at 37°. No. 23.

Aged 16 to 17 months. Doses of 2.5, 5 and 10 cc. None of these afforded any protection.

Orange juice preserved with the rind oil does not therefore appear to retain its anti-scorbutic potency at 37° any better than does lemon juice preserved in the same way or with sulphite.

III. PRESERVATION OF CHILLED FRUIT, ORANGES AND LEMONS.

At the request of the Food Investigation Board, some tests have been carried out in order to determine the extent to which the anti-scorbutic principle is retained when fruit is preserved by the chilling process, *i.e.* cold storage at temperatures which do not permit of freezing.

Carefully selected samples of oranges and lemons have been kept in cold storage at a temperature of 2.5 to 5.4°. The fruits were wrapped in paper to prevent contact with one another and packed in crates. They were examined periodically and any found to be soft or mouldy were removed. Fluctuations in the temperature of the storage chamber were registered daily by means of a self-recording apparatus. The highest maximum temperature recorded during the period of storage was 5.4° and the lowest minimum was 2.5°.

After periods of several months, a supply of fruit was removed for testing. The juice was prepared as described for fresh fruit every other day, and the fruit and juice were kept in the Lister Institute refrigerator during the period of experiment.

A. Oranges. No. 24. The fruit was put into storage in May 1919 and tests were begun in October, 1919, after five months' storage. At the periodic examinations between these dates, 44 % of the original fruit had been rejected. When the testing was begun 96 oranges were available of which only 11 were perfectly sound; all the rest were partly bad but their juice was tested.

Experiments were made with 5 cc., 3 cc. and 1.5 cc. daily doses and were continued for 75 days only. Two guinea-pigs were used for each test. With all three doses complete protection from scurvy was obtained.

After five months' cold storage of the fruit therefore, the anti-scorbutic potency of orange juice is not appreciably impaired, since a daily dose of 1.5 cc. will still protect a guinea-pig from scurvy (minimum dose of fresh orange juice 1.5 cc. daily, see p. 90).

B. Lemons. No. 25. The lemons were first tested in January 1920, after nine months' cold storage. During this period they became very mouldy and at different times 108 out of the original 238 were discarded. At the time of testing none was completely sound.

Experiments were made with 1.5 cc., 2.5 cc. and 5 cc. daily doses. With the 1.5 cc. daily dose severe scurvy terminated the experiment after 32 days. The 2.5 cc. daily dose gave a similar result. Of two animals receiving 5 cc. daily, one died from lung haemorrhage after 28 days, distinct scurvy being present; the other animal showed sore and painful joints by the 25th day and died from severe scurvy after 51 days.

Thus even the 5 cc. daily dose was practically useless, whereas in the fresh state a 1.5 cc. daily dose of lemon juice affords complete protection from scurvy. The test is not satisfactory because the fruit was not prevented from becoming mouldy. It was in much worse condition and four months older than were the oranges, though neither was in a condition which would ordinarily be regarded as edible. It is probably therefore safe to say that the juice of oranges and lemons, which are preserved by chilling, does not lose appreciably in anti-scorbutic value as long as the fruit is fit for food, but it cannot apparently be kept fit for food in a "chilling" cold store for more than a very few months.

CONCLUSIONS AND SUMMARY.

- 1. Further experimental evidence is advanced as to the inferiority of lime juice to lemon and orange juice as an anti-scorbutic.
- 2. The minimum daily doses of the juice of these three Citrus fruits, needed to protect a guinea-pig from scurvy are established as follows:

Lemon ... 1.5 cc. Orange ... 1.5 cc. Lime ... 5.0 cc.

These values are used as a basis for comparing the keeping properties, as anti-scorbutics, of the juice of oranges and lemons. The juice is kept for varying times at different temperatures, and in the case of lemon juice preservation with sulphite and with the rind oil is tested, and in the case of orange juice preservation with the rind oil.

- 3. Preservation with sulphite appears to be satisfactory at a low temperature (about 0°) but at room temperature it seems uncertain and at 37° it is useless, the juice becoming bad in the gross sense as well.
- 4. Preservation with the rind oil, in case of lemon, is satisfactory and reliable at about 0° and at room temperature. With oranges preservation by this method at 0° was not tried but at room temperature the result was satisfactory. At 37°, both for oranges and lemons, it is as unsatisfactory as is preservation with sulphite, though the juice was not as unpalatable as was the juice preserved with sulphite.

Consequently for 0° and for temperatures about English room temperature, preservation with the rind oil is suggested as the most reliable. There is loss, but even after two years it is not very great.

- 5. Preservation at 37° has not so far been secured by any method; it is possible that could preservation in the gross sense be secured, the antiscorbutic property might also be better preserved; it is suggested that this might be attained by boiling the juice first to render it sterile. It has been shown by Delf [1920] that to heat orange juice for an hour at 100° does not materially diminish its anti-scorbutic potency.
- 6. Experiments are also described on the preservation of oranges and lemons, kept in a chilling (not freezing) cold store; the results are not quite consistent but it appears probable that the anti-scorbutic property is not seriously diminished so long as the fruit is edible.

In conclusion it is my pleasant duty to record my gratitude to Miss Hume, who when I was obliged to resume my university work, undertook the task of final preparation of this paper for the press. Thanks are also due to Miss H. Henderson Smith and to Miss S. Rutherford for assistance in feeding the animals.

A part of the cost of the research was defrayed by the Medical Research Council to whom thanks are also tendered.

REFERENCES.